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Report of the Panel on Basic Sleep Research

Introduction

One-third of our lives is spent in sleep. No other single behavior occupies so much of our time, yet few other behaviors are so mysterious. Sleep, the gentle tyrant, forces us to seek our beds each night, only to arise each morning with scant recollection of the preceding hours. The great minds of our civilization—Aristotle, Shakespeare, Picasso, and Freud among many others—have sought meaning and purpose in these sleeping hours, yet no good answers exist to the questions of why we sleep or how.

For many Americans pathological daytime sleepiness is a constant companion, while for others nocturnal sleep is frustratingly elusive. Disorders of sleep are among the most prevalent complaints in our society. Nearly everyone has suffered from transient insomnia, associated with conditions such as jet lag or stress, but millions of persons suffer each night for weeks or months with chronic insomnia. For the hundreds of thousands of people with sleep apnea syndrome or narcolepsy, excessive daytime sleepiness interferes with driving, employment, and family and social life.

Tremendous growth in the basic neurosciences in the past two decades has generated a scientific framework for understanding the basic mechanisms and functions of sleep. Much progress has been made and many of the fundamental questions are now being actively investigated. This report summarizes an Institute of Medicine meeting on basic sleep research. Its purpose is to highlight recent progress in the understanding of sleep, and to indicate areas and methodologies of sleep research that hold promise for further advances.

A Brief Synopsis of Sleep Structure

The electroencephalogram, or EEG, has long been the primary means of studying sleep. The activity of neurons in the cerebral cortex generates electrical fields strong enough to be detected through the skull by sensors placed on the scalp. These small (microvolt) electrical signals are amplified and filtered to produce EEG recordings. Although the EEG is a crude way to determine brain activity (similar to figuring out what is happening in a football game by putting microphones outside the stadium), it has proved a remarkably useful tool for studying the basic structure of sleep.

Sleep is not a uniform state. In sleep laboratories and clinics, a number of variables are measured during a typical night's sleep. These include the continuous recording and graphing of EEG, eye movements, and muscle tone in a process known as polysomnography. Humans, like nearly all mammalian species, exhibit two major states of sleep: rapid eye movement (REM) sleep and nonREM sleep. The majority of a night's sleep is spent in nonREM or slow-wave sleep, which is divided into Stage 1 through Stage 4 according to EEG waveform patterns (Figure 1A). As sleep onset approaches, the low amplitude, fast

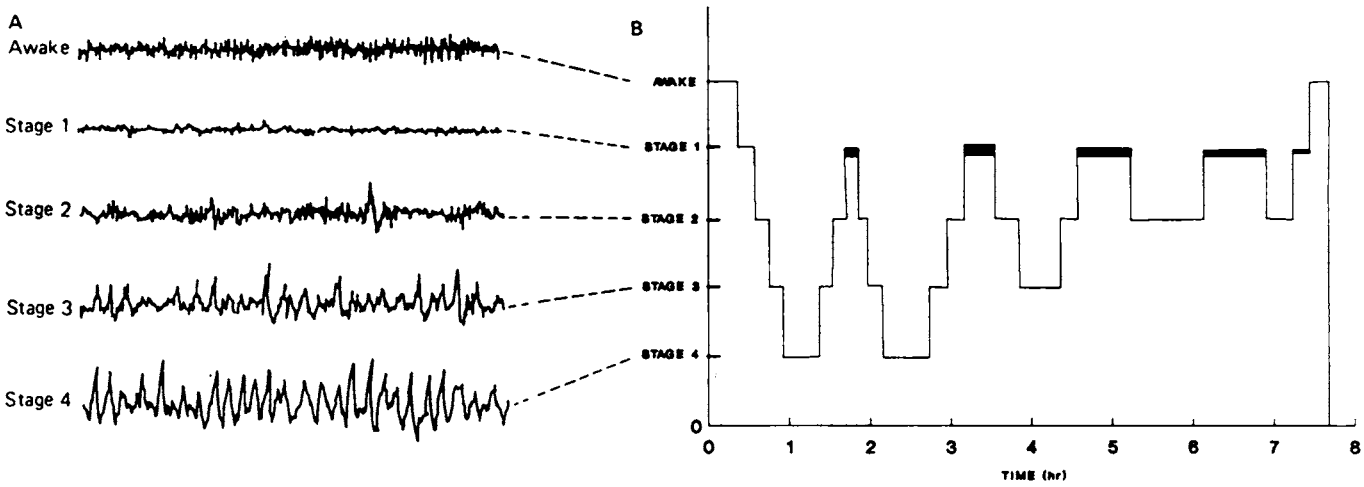

Figure 1 A. Examples of the EEG patterns during the various stages of sleep, each trace represents about 30 seconds of recording. B. A typical night's sleep pattern in an adult. REM sleep is indicated by black bars. Notice that the period of time spent in REM sleep lengthens with successive sleep cycles, as does the amount of Stage 2 non-REM sleep. (Figure adapted from: Kandel, E.R., and Schwartz, J.H. (eds.) *Principles of Neural Science, 2nd ed.* New York: Elsevier Science Publishing Co., Inc., 1985, p. 650.)

frequency EEG of alert wakefulness yields to slower rhythms. Stage 1 sleep is a brief transitional phase between wakefulness and "true" sleep, characterized by mixed amplitude, mixed frequency EEG patterns. During Stage 2 sleep, there are episodic bursts of rhythmic, 14-16 cycles per second waveforms in the EEG, known as sleep spindles, interspersed with occasional high amplitude, slow waves. Stages 3 and 4 are defined, respectively, by lesser and greater occurrence of high amplitude, slow (0.5-4 cycles per second) waveforms, called delta waves.

The low voltage fast EEG pattern of REM sleep is in marked contrast to delta sleep in that it is most similar to the EEG pattern of active wakefulness (Figure 1A). In addition, REM sleep is characterized by bursts of rapid eye movements and by loss of muscle tone in certain major muscle groups of the limbs, trunk, and neck. Dreaming is associated with REM sleep.

There is a predictable pattern of shifting between one sleep state and another during a typical night's sleep (Figure 1B). As the night begins, there is a stepwise descent from wakefulness to Stage 1 through to Stage 4 sleep, followed by a more abrupt ascent back toward Stage 1. However, in place of Stage 1, the first REM sleep episode usually occurs at this transition point, about 70-90 minutes after sleep onset. As the night progresses, this cycle repeats, but with less and less delta sleep and longer REM sleep episodes. Approximately 20-25 percent (or 90-100 minutes) of total sleep time is spent in REM sleep. This pattern of sleep is influenced by age. For example, a newborn spends up to 50 percent of its total sleep time of 16 hours in REM sleep. The greatest amount of time spent in delta sleep is in early adolescence. Through adolescence and adulthood, the amount of REM sleep remains fairly constant. However, total sleep time typically decreases with age and Stage 4 sleep often disappears entirely by age 50 or 60.

A number of physiological and psychological correlates have been determined for each of the general EEG states. The delta-wave activity (Stage 3 and 4) seen in the first part of the night corresponds roughly to the deepest sleep, the sleep from which it is most difficult to arouse the subject. Persons awakened from this state show marked diminution in their cognitive abilities—they are confused and groggy. Deep, slow-wave sleep is also marked by slow, regular breathing and a paucity of movement. Longer periods of wakefulness prior to sleep increase the amount of delta sleep.

REM sleep, in contrast, has a number of physiological correlates normally associated with wakefulness and activity: rapid eye movements, phasic twitches of the musculature, activation of brain centers involved in vision, hearing, and muscle control, and moderately high brain metabolic rate. This complex state of sleep has been shown to be highly correlated with the dreaming process. Sleep studies in humans have shown that, when subjects are awakened during REM sleep, 80 percent of the time they report that they had been dreaming. Some dreaming is also reported on awakenings from nonREM sleep. Thus, most, but probably not all, dreaming occurs during REM sleep.

There are a number of biochemical changes involv-

ing the release of pituitary hormones that occur during sleep. For example, there is an increase in growth hormone release just at the onset of delta sleep. Another hormone, prolactin, is released throughout a night's sleep. During early adolescence, luteinizing hormone, important to the stimulation of maturity in the sex organs, is released during sleep. Sleep onset inhibits the release of thyroid stimulating hormone and, to a minor degree, cortisol secretion.

A number of disorders of sleep can be understood within the architectural framework derived from EEG recordings. Narcolepsy is a disease that affects an estimated 100,000 people in the United States. It is characterized by the symptoms of excessive daytime sleepiness, cataplexy (sudden brief loss of muscle tone, usually brought on by laughter, anger, or sudden emotion and sometimes leading to the collapse of the individual), and hallucinations accompanying sleep onset. Narcolepsy results from an inappropriate, uncontrolled expression of REM sleep or its components during wakefulness. The symptoms of cataplexy and hallucinations reflect the muscle atonia and dreaming that normally occur in REM sleep. The diagnosis of narcolepsy is relatively straightforward, because narcoleptic patients typically enter REM sleep immediately at sleep onset, skipping the initial slow-wave sleep stages. Thus, in narcolepsy, the REM latency (time from the onset of sleep until the onset of the first REM period) is close to zero rather than the normal 70-90 minutes. A short REM latency, without cataplexy or hallucinations, has also been discovered in depression and certain other major psychiatric disorders.

Control of Sleep and Wakefulness

Considerable progress has been made in the past decade in understanding the factors that control the timing and structure of sleep. Fifteen years ago, no model of sleep was capable of predicting when sleep would begin in the absence of time cues, and how long sleep would last depending upon when it began or how long a period of wakefulness preceded sleep onset. Now, however, several areas of investigation have demonstrated that two dominant processes determine the timing and structure of sleep: circadian (daily) rhythmicity and homeostasis. To simplify these determinants, sleep timing and duration are controlled by when we sleep in relationship to an underlying circadian clock and how much sleep "debt" (how long one has been awake) has accumulated prior to sleep. It has become possible to model mathematically these two processes and to predict with some accuracy the timing and duration of sleep under many conditions.

Animals and people living without any external time cues continue to function rhythmically. People living in time isolation, for instance, adopt a roughly 25-hour day. A number of physiological functions are synchronized to this day-length, and seemingly to each other, including sleep and wakefulness, core body temperature, and adrenocorticotropin hormone and cortisol secretion; such rhythms are called circadian (about a day) rhythms. The maintenance of this rhythmicity in the absence of environmental cues suggests the presence of some internal clock within the organism that can keep time on its own. In 1972, it was discovered that such a clock mechanism resides in a pair of very small brain nuclei called the suprachiasmatic nuclei (SCN), located within the anterior hypothalamus just above the crossing of the optic nerves. Animals in which these nuclei are destroyed no longer maintain 24-hour rhythmicity of sleep and wakefulness in the absence of light and dark cues. That is, these animals show short bursts of sleep and wakefulness randomly distributed over the 24-hour day. Transplantation of cells from fetal suprachiasmatic nuclei to an animal altered in this way replaces the lost neurons with new cells and restores normal circadian rhythmicity.

Although the circadian clock exerts a powerful influence on the timing of sleep and wakefulness, circadian sleep-wake patterns need not be synchronized with other circadian rhythms under some circumstances. For example, most people kept in time isolation—in special apartments where they are deprived of time cues such as clocks and solar light—will eventually dissociate their sleep-wake cycle from their body temperature cycle, a process called spontaneous internal desynchronization. For example, the average cycle length of sleep and wakefulness might be 32 hours (the time from the beginning of one sleep episode until the next one), but the average length of the temperature cycle might be 25 hours (the time from one peak until the next). Under these circumstances, the individual no longer goes to sleep at the same phase position of the daily temperature cycle, normally about midway between the late afternoon high point to the late night low point. Rather, the individual may go to sleep at any point of the temperature curve. Analysis of data from people kept in internal desynchronization has led to the development of a mathematical model suggesting that the control of the sleep and wakefulness cycle arises from the interaction of two oscillatory processes—a "strong" one controlling the temperature cycle, and a "weak" one controlling the onset of sleep. This model predicts the timing of sleep and wakefulness. Such a model also explains the finding, for instance, that the length of time a person will stay asleep is determined mostly by the

time or phase of the circadian cycle when sleep begins, and to a lesser extent by the amount of time awake before falling asleep. Furthermore, sleep is more likely to occur near either the low point of the body temperature curve or shortly before the high point (the "siesta" period).

The close relationship between the circadian and sleep-wake systems now permits the rational manipulation of the timing of sleep and wakefulness. Light has long been known to be a strong synchronizer of circadian systems in animals; recently, preliminary studies have shown that properly timed exposure to bright light can rapidly reset the human circadian pacemaker to any desired phase within two or three days. This finding has implications for the treatment of early awakening in the elderly and in delayed sleep phase insomnia. In addition, it may lead to treatments to help workers adapt to a night shift and to help travelers avoid jet lag.

Certain critical features of sleep, however, are not explained by circadian control. Why should lack of sleep make one feel sleepy the following day, if sleep is just turned on and off by a clock in the brain? In animals with intact suprachiasmatic nuclei, the timing of sleep and wakefulness is dominated by the circadian timing system; however, when this system is removed (by destruction of the SCN), the length of time an animal sleeps is dependent on how long the animal has been awake previously. Sleep seems to be compensatory in these animals. Similarly, animals selectively deprived of REM sleep will make up the lost REM sleep on subsequent nights, something that is not predicted by a strictly circadian model of sleep control. Such compensatory mechanisms are known as homeostatic processes. These processes maintain equilibrium in a number of physiological systems from blood sugar regulation to body temperature control.

Several homeostatic models of sleep have been proposed. One of the more compelling of these models proposes that the continual accumulation of a chemical sleep-inducing factor during the day produces drowsiness eventually leading to sleep. Although there are several biochemical candidates, which will be discussed later, a specific sleep factor has not yet been identified. However, there are experimental data that support the existence of such a factor. For example, the amount of delta sleep increases in animals following the administration of one of the candidate biochemicals. Further, the amount of delta sleep also increases with prior sleep deprivation, supporting the concept that there is a build-up of some substance during time spent awake.

These phenomenological models each have impressive predictive power, and it has become clear that both clock-driven and homeostatic mechanisms ultimately determine the timing and quality of sleep. Three major challenges now confront researchers interested in the control of sleep and wakefulness. First, the homeostatic and circadian influences need to be integrated into a single functional model that can describe both the timing of sleep and its quality. Certain features of sleep not presently represented in either model (for instance, the tendency to fall asleep in the middle of the afternoon) need to be explained in a unified manner. Second, the neurophysiological and biochemical mechanisms that generate the observed sleep/wake behaviors must be elucidated. Third, the models need to predict the timing and quantity of both nonREM and REM sleep. Most of the theoretical modelling so far has focused on the timing and duration of sleep and wakefulness with the exception of the prediction of timing and amount of REM sleep at various circadian phases by the limit cycle reciprocal interaction model, whose neurophysiological features are discussed later.

Sleep Mechanisms

Biochemical Mechanisms of State Control: Sleep-Inducing Factors

The presence of sleep-inducing factors has been implicated experimentally since the turn of the century. In an experiment conducted in 1913 by Pieron, dogs were deprived of sleep and a small amount of cerebrospinal fluid (the fluid surrounding the brain) was removed and injected into the cerebrospinal fluid of a non-deprived animal. The non-deprived animal's sleep time increased, an observation that has been repeated several times. To date, only one substance has been found to vary in concentration in the cerebrospinal fluid with slow-wave sleep, interleukin-1. Interleukin-1 is a molecule that was originally found in cells of the immune system and later in certain brain regions. Interleukin-1 appears to induce nonREM sleep. Several other substances, including growth hormone releasing factor and vasoactive intestinal peptide, have also been implicated in the regulation of sleep. Still other molecules, muramyl peptides, have been found in the brains of sleep-deprived animals. These same muramyl peptides serve as building blocks of bacterial cell walls that are recognized by the host's immune system as a signal of a bacterial infection. Muramyl peptides also stimulate the release of cytokines, which are potent chemicals released from cells of the immune system and from certain cells in the brain. Interleukin-1 is such a cytokine; another, interferon-2, also induces sleep. The mechanism by which

cytokines induce sleep is not known. However, in addition to possible direct effects, cytokines are also known to stimulate the metabolism of molecules called prostaglandins and one of these, prostaglandin D2, has been shown to enhance sleep. Such findings have stimulated increased research into the relationship between the functions of the immune system and sleep.

It has been noted anecdotally since antiquity that drowsiness is common in infectious illness. Recently, the actual amount of sleep associated with bacterial infection has been quantified in animals. However, sleep changes associated with viral infections have not yet been quantified. Thus, the precise role of molecules synthesized by the immune system in sleep and the possible effects of sleep on immune system function are now areas of active investigation. Such studies may contribute to an expanded understanding of the physiological processes underlying infections and may suggest new strategies for therapeutic intervention.

Although the identification of endogenous substances that are able to induce sleep does not enable the conclusion that they are involved in the normal regulation of sleep and wakefulness, their discovery is nonetheless important for several reasons. First, it provides a rationale for searching for other substances that may be involved in the daily regulation of wakefulness and sleep. Second, these substances can become powerful tools for research in that they enable investigators to induce sleep in an animal at will. For example, the effects of the circadian system on the expression of sleep could be delineated by the induction of sleep at times of day when sleep is normally prohibited by the internal clock and by the subsequent study of the quality and timing of that sleep. Third, molecular biological techniques (e.g., DNA probes for these substances and their receptors) can be used to determine how, where, and when their gene transcriptions and translations occur in the brain as a function of sleep. Finally, the existence of endogenous sleep-inducing substances may help in the development of therapeutic agents that would induce a more natural sleep with fewer side effects than currently used drugs.

The Neurophysiology of Slow-Wave Sleep

Electrophysiological recording of neuronal activity has been applied to the sleeping brain for more than 35 years and has produced a wealth of information on the cellular mechanisms of sleep and wakefulness. These studies, however, depended on the knowledge gained from anatomical investigations that sought to identify specific brain areas important in sleep. Early studies in cats, in which the brainstem[1] was transected at different levels by knife cuts and subsequent sleep was monitored, implicated several regions as important to the control of transitions from sleep to wakefulness. These studies found that, if cuts were made at the junction of the spinal cord and the brainstem, the animals would exhibit a normal sleep/wake cycle. In contrast, if the cuts were made at a higher level of the brainstem (the midbrain region), the cats exhibited an EEG pattern typical of slow-wave sleep. Later, other investigators found that cuts made between these levels, at the middle of the pons, produced animals that were continuously awake. Still later studies demonstrated that electrical stimulation of the brainstem reticular formation, a network of connected brain areas that run throughout the core of the brainstem, could "activate" the EEG in a pattern consistent with arousal. These studies led to the concept that an "ascending reticular activating system" controlled sleep and wakefulness. More recent studies have modified this concept to include certain regions that are specialized parts of the reticular formation. Of particular importance is the discovery of functionally important neurons that contain the neurotransmitter acetylcholine and are located at the pons-midbrain junction. These neurons have an important role in forebrain and thalamic activation in REM sleep and in waking, and may, indeed, be required for the induction of REM sleep. Stimulation of these regions (part of a more general "ascending activating system") produces an arousal state in animals and disruption of the connections between these systems and forebrain in animals produces a state with a slow-wave, sleep-like EEG.

It was initially thought that the neurotransmitter serotonin might be the messenger of slow-wave sleep. However, electrical recordings of the serotonin containing neurons in the raphe nuclei showed that the electrical activity or firing rates of these neurons decreased in slow-wave sleep and this theory was consequently abandoned. The precise cellular and molecular mechanisms by which activity in the ascending activating system is "turned off" to allow slow-wave sleep remain obscure, but, as discussed, may involve humoral factors. Also, there is evidence that destruction of certain regions in the anterior hypothalamus and basal forebrain prevents slow-wave

1 The brainstem is a term that describes the gross anatomy of the brain from the top end of the spinal cord, at the base of the skull, to the thalamus, located between the cerebral hemispheres. The brain stem is divided into regions as one moves from the spinal cord to higher levels. These regions are the medulla, pons, and midbrain.

sleep, indicating that there may be some active slow-wave sleep generators.

There has been great progress in understanding the mechanisms by which the changes in neuronal activity in the thalamus (the way station between the more primitive brainstem areas and the cortex) occur as a function of sleep state. The cortex is the source of conscious sensation and thought in the brain; our relative unawareness of the external environment during sleep may result from selective blockage of sensory information reaching the cortex. The site of this blockade is now thought to be the thalamus. Nevertheless, sleep should not be equated with coma or anesthesia. For example, the sleeping organism continues to monitor the environment in order to respond to threats, such as a crying baby or the smell of smoke. Changes in thalamic function can be seen, albeit indirectly, in the EEG, where so-called spindle activity of short bursts of regular 7-14 Hz periodicity (generated by the thalamus) are prominent in nonREM sleep.

Through use of single neuron recordings from neurons in the thalamus, combined with stimulation or destruction of specific thalamic inputs, three factors have been discovered to be fundamental to the activity of the thalamus in sleep. First, the intrinsic, biophysical properties of thalamic neurons are changed in sleep. In particular, the rate of conductance of ions across their membranes changes and, thus, the neurons' ability to conduct the electrochemical impulses (action potentials) needed to affect other neurons is altered. The result of such changes is that inputs to the thalamus that would have been transmitted to the cortex during wakefulness are not relayed to the cortex during sleep. Second, cells in one thalamic nucleus, the reticular thalamic nucleus[2], have been found to be the pacemaker of the observed spindles. These cells are known to contain the inhibitory neurotransmitter, gamma-aminobutyric acid or GABA. Based on extra- and intracellular recordings of these neurons, a model has emerged that suggests that the rhythmic firing of reticular thalamic neurons leads to rhythmic inhibition of the neurons connecting the thalamus to the cortex. This model provides a cellular explanation for the phenomenon of spindling, a generalized feature of nonREM sleep; the function of the spindles, however, remains to be determined. Finally, the model may also help explain how one type of arousal, evidenced by electrical activation of the cortex, is generated in the thalamus. It has been demonstrated that electrical stimulation of certain regions in the ascending activating system produces arousal and that a direct effect of this stimulation is the removal of the inhibition exerted by the pacemaker cells of the reticular thalamic nucleus. As before, these effects are the result of altered ion conductance in the thalamic neurons. Despite these findings, the thalamus may not be the only structure responsible for cortical activation, because cortical activation can occur when the thalamus has been destroyed. Nevertheless, these studies demonstrate how some features of sleep can be described in a neurophysiological framework. Although these studies have elucidated some of the cellular phenomena of nonREM sleep, the mechanisms of sleep onset, the interactions of the circadian system (suprachiasmatic nuclei) with the ascending activating system and other arousal- and/or sleep-producing brain centers, and the neurobiology of the homeostatic nature of sleep remain unresolved.

The Neurophysiology of REM Sleep

Narcolepsy is characterized by sleep onset REM episodes and the inappropriate appearance of physiological and psychological components of REM sleep during wakefulness. For example, the muscle atonia and dreaming typical of REM sleep results in cataplexy and hypnogogic hallucinations, respectively. Since narcolepsy is a disorder of REM sleep, it has also stimulated a great deal of basic physiological studies on the regulation of REM sleep.

It has not been possible to completely eliminate nonREM sleep by destruction of specific brain regions. However, REM sleep has been markedly reduced by ablation of a specific region of the pons. By recording the electrical activity of neurons in the intact pons, near this region, several populations of neurons, which are active during and may promote REM sleep, have been identified. These cells contain the neurotransmitter, acetylcholine. Other neurons, located nearby, decrease their activity during REM sleep.

During the past twenty-five years a great deal of information has been gathered indicating that acetylcholine elicits and maintains REM sleep and its individual physiological components, such as the low amplitude, fast frequency EEG, loss of muscle tone, and bursts of rapid eye movements. REM sleep has been triggered in animals by the administration of drugs that mimic acetylcholine (cholinomimetics), such as carbachol, oxotremorine, and bethanechol, into the medial pontine

2 The term "reticular" is an anatomical description referring to the heterogeneous appearance of certain brain regions that contain neurons and axon fiber tracts mixed together (as opposed to a compact, dense collection of neurons). The term does not imply that the reticular activating system and the reticular thalamic nucleus are part of one another.

reticular formation. Acetylcholine, like many neurotransmitters, exerts its action on neurons by first binding to proteins, called receptors, on the cells' surfaces. Each of these receptors recognizes specific neurotransmitters. Acetylcholine is recognized by and binds to two types of receptors, a muscarinic acetylcholine receptor and a nicotinic acetylcholine receptor, that were identified by their differential activation by either the drug muscimol or nicotine. The muscarinic, or M, receptor is particularly implicated in the effects of acetylcholine on REM sleep and its components. More recently, drugs that have relatively selective effects on a specific subtype of muscarinic receptors (the so-called M2 receptor), such as cisdioxolane and oxotremorine-M, have been shown to induce REM sleep, while drugs active at the M1 muscarinic receptor subtype have no effect. Furthermore, REM sleep can be facilitated in humans by administration of cholinomimetic drugs, including physostigmine, arecoline, RS 86 (an experimental muscarinic agonist), and pilocarpine. When subjects were awakened from physostigmine-induced REM sleep, they reported dreams that were entirely similar to dreams reported from spontaneous REM periods. In support of evidence that muscarinic receptors mediate REM sleep, the administration of drugs that block the muscarinic receptor, such as scopolamine or atropine, inhibits spontaneous REM sleep in both animals and humans and blocks the REM sleep-inducing effects of cholinomimetic drugs.

Recent studies suggest that the cholinergic neurons important for REM sleep originate in the laterodorsal tegmental nucleus and the pedunculopontine nucleus, located in the pons and lower midbrain. These cholinergic neurons project to three regions essential for REM sleep components: the thalamus, a region that promotes EEG activation during REM sleep; the medial pontine reticular formation, a region where administration of cholinomimetic agents readily induces REM sleep; and the medulla, a region important in the production of muscle atonia during REM sleep. Complete and selective destruction of these cholinergic neurons is not technically possible at this time, but relatively selective destruction with the toxin, kainic acid, greatly reduces REM sleep in cats. All of these observations strongly indicate an important role for cholinergic mechanisms in the regulation of REM sleep.

If cholinergic neurons promote REM sleep, it is possible that noradrenergic (norepinephrine-containing) neurons in the locus ceruleus and serotonergic neurons in the dorsal raphe inhibit it. Many neurons within these two nuclei slow down or even stop firing just before and during REM sleep. In some studies, REM sleep has been promoted by the administration of drugs that inhibit or reduce the effects of norepinephrine. Indeed, one theoretical model for the control of REM and nonREM sleep, the reciprocal interaction model, has postulated that cholinergic, REM-promoting neurons interact directly with noradrenergic and serotonergic, REM-inhibiting neurons, to control the oscillation between REM and nonREM sleep. This model has been of great heuristic value, but further research will undoubtedly be necessary before a complete theory emerges.

As an interesting illustration of how basic science leads to new clinical discoveries, it has been known for over fifteen years that, in cats, destruction of an area just below the locus ceruleus eliminates muscle atonia during REM sleep. These animals walk about and engage in complex behaviors during REM sleep. A recently described human clinical disorder, the REM sleep behavior disorder, has features analogous to this animal preparation. In the human disorder, the individuals retain normal muscle tone during REM sleep and perform actions that correspond to their dreams. For example, the patient might run across the bedroom into the dresser while dreaming about playing football. However, because no autopsy material is available from any of these patients, it is not known whether or not they have neuropathological abnormalities in the reticular formation below the locus ceruleus.

Of the three major arousal states (REM sleep, slow-wave sleep, and wakefulness), the cellular anatomy and physiology underlying the generation of REM sleep is best understood. Knowledge regarding the location and chemical identities of neurons important in the generation of REM sleep has allowed investigators to begin to determine the molecular events underlying the observed cellular physiology. It is now clear that the sleep state of the animal modulates the intrinsic, biophysical properties of individual neurons. This has been best described in studies of the inhibition of muscle activity during REM sleep. As mentioned earlier, there is no tonic muscle tone during REM sleep; similarly, reflex activity (such as the clenching of the jaw following stimulation of the trigeminal nerve) is suppressed in adult animals during REM. Intracellular recordings of the electrical activity of the neurons that control muscle movement (motor neurons) during natural sleep have shown that muscle tone suppression during REM sleep results from an inhibition of motor neurons. This inhibition reduces the excitability of motor neurons, making them less likely to discharge action potentials. Recently, the inhibitory neurotransmitter, glycine, has been found to be responsible for these alterations. Impulses that are generated in a particular set of reticular formation neurons below the locus ceruleus in the pons and relayed via inhibitory regions of the reticular formation

in the medulla to motor neurons are responsible for this characteristic motor neuron inhibition during REM sleep. This inhibition is very powerful and suppresses most output despite the simultaneous, powerful excitatory inputs that impinge on the motor neuron in REM sleep.

Similar intracellular recordings of other neurons active during natural REM sleep have shown that their increased excitability and action potential production during REM sleep result from a modulation of the normal membrane potential (difference in charge, or distribution of ions, between the inside and outside of a neuron). Such REM sleep activation of particular pontine reticular neurons produces the rapid eye movements. Recently, it has also become possible to study the physiology of pontine neurons through the use of brain slice techniques. In this case the pontine region responsible for REM can be removed from the brain, put into a chamber, and bathed with nutrient media. This procedure allows for the intracellular recording of neurons under a variety of experimental manipulations. Such studies have identified several subpopulations of cells in the REM-generating regions on the basis of their differing intrinsic electrophysiological properties and have been able to predict how REM sleep-induced changes in membrane potential will affect the firing characteristics of the neuron by modulating these intrinsic properties. For example, it has been found that the membrane depolarization present during REM sleep (in which the inside of the neuron becomes less negative in charge) inactivates a molecular channel conducting calcium ions across the membrane and results in a decreased tendency for the neuron to fire in bursts. In addition, the brain slice preparation has shown how acetylcholine acting on non-M1 muscarinic receptors mimics the increased excitability and membrane depolarization normally recorded in reticular neurons in REM sleep, thus supporting the critical role of this neurotransmitter in REM sleep production.

Our current understanding of the physiology of REM sleep is applicable to narcolepsy. In the past decade, it was found that certain dogs naturally become narcoleptic. This animal model of narcolepsy has permitted a more rigorous investigation of the disease than might otherwise be possible. For example, the administration of acetylcholine-like substances has been shown to generate cataplexy in narcoleptic dogs. Further work has also shown that this cataplexy is blocked by the action of norepinephrine on a specific receptor molecule, the alpha-1b adrenergic receptor. In addition, examination of the brains of narcoleptic dogs has shown increased levels of muscarinic acetylcholine receptors in the brainstem reticular formation compared with control animals. Understanding narcolepsy to this level of biochemical detail has allowed, for the first time, the beginning of a systematic evaluation of pharmacologic agents for their beneficial effects in human disease. Therefore, it is anticipated that these studies should have immediate application to the treatment of human narcolepsy.

The Evolution, Ontogeny, and Function of Sleep

Despite a wealth of knowledge uncovered in the past 15 years about the architecture, control, and mechanisms of sleep, the function of sleep is still unknown. Both REM and slow-wave sleep have been reported in all but a few primitive and marine mammals, and sleep-like behaviors have been reported in birds, reptiles, fish, and invertebrates. The time an animal spends asleep is a function of its size; bats sleep about 20 hours a day; whereas elephants sleep less than five. This relationship suggests that one function of sleep is to conserve energy, because metabolic rates are higher in small animals and lower in large animals. However, other data argue against such a simple explanation. When the relationships of body size and metabolic rate are statistically separated, animals with high metabolic rates tend to sleep less. Also, metabolic rate during sleep is only about ten percent less than during quiet wakefulness. Further, induced changes in metabolism have little effect on total sleep. Thus, the role of sleep is probably not only to save energy.

One way to assess the function of sleep is to assess the effects of sleep deprivation. Long-term sleep deprivation has been difficult to perform, but recent progress in computerized EEG analysis has allowed the construction of automated sleep-deprivation devices. Rats subjected to total sleep deprivation survive an average of three weeks, and rats subjected to REM deprivation survive an average of five weeks. A most striking change in sleep-deprived rats is a large increase in energy expenditure. Food intake doubles, while body weight declines. Since body temperature declines in spite of increased heat production, excessive heat loss is indicated. Thus, sleep may play a role in normal thermoregulation.

One theory of the function of REM sleep relates to brain development. Although, for any age group, the architecture of normal sleep is relatively consistent, it changes markedly over the lifetime of the individual. REM sleep occupies a much larger percentage of the infant's sleep than the adult's, and total time spent in REM sleep is much higher for the infant as well. The features of REM sleep include strong cortical activation of the central nervous system and simultaneous suppression of actual muscle activity. It is known in several systems (particularly in the development of the visual system) that synaptic activity is required for normal neuronal

development. The developmental theory of REM sleep suggests that REM provides a means for generating the brain activity required for development without placing the infant at risk of injury due to the muscular activity that would occur in wakefulness. Although teleologically satisfying, this developmental theory of sleep has been difficult to test, because it is difficult to deprive fetal and infant animals of REM sleep. Recent findings of the presence of a particular kind of neurotransmitter receptor, the NMDA excitatory amino acid type, throughout the pontine reticular formation may offer a promising pathway for investigation of neural growth and plasticity. Activation of these receptors, likely quite prominent in REM sleep, leads to calcium ion influx and the associated increase in intracellular calcium has been shown to be important for regulation of neural growth and plasticity in a number of brain regions.

Other theories of sleep function have postulated that sleep plays a role in protein synthesis, information processing and memory storage, cognitive function, enforcement of rest, and behavioral adaptation. The enigma of sleep's purpose remains one of the great unsolved questions of sleep research; without a clear-cut functional role for sleep, the types of structure-mechanism-function reasoning that have been so powerful in guiding research in all other aspects of physiology and behavior are not available. As more is learned about the control and mechanisms of sleep, the selective advantages of sleep as well as the reasons for its conservation throughout mammalian evolution may become apparent. In addition, as more is learned about sleep function, we may be able to make more sense out of the diverse physiological changes that occur during sleep.

Physiological and Pathological Correlates of Sleep

Thus far this report has been concerned with the processes and mechanisms that generate sleep. Once induced, sleep profoundly influences a broad range of physiological functions and can generate pathologies not seen in wakefulness. Examples of such altered physiologies can be found in the cardiovascular and respiratory systems. It is now also apparent that certain psychiatric diseases are associated with deviations from normal sleep.

Somatic Correlates of Sleep

The effects of sleep on respiratory physiology are of immense clinical importance. During sleep, the upper airway musculature relaxes and the airway narrows, sometimes leading to snoring and, in some persons, to obstructive sleep apnea syndrome. In this disorder, the airway closes and breathing ceases for periods up to two minutes. Arterial oxygen saturation levels can fall to below sixty percent, which is less than the normal venous oxygen saturation. The night's sleep becomes punctuated with numerous arousals, causing patients with sleep apnea syndrome to be pathologically sleepy during the day - they will fall asleep in any low stimulus environment at any time of day in less than five minutes. Such a propensity is especially dangerous when they are, for example, behind the wheel of a car.

Much progress has been made in the last ten years in understanding the changes in respiratory function that occur with sleep. A major change in respiratory physiology during sleep involves the neurons that control the muscle tone of the pharynx (the upper airway motor neurons). It has been discovered that, in males particularly, these neurons are inhibited during sleep, leading to a relaxation of the muscles in the pharynx. This, in turn, increases tenfold to 100-fold the resistance of the upper airway to airflow, and greatly aggravates any anatomic factors (such as excessive tissue around the neck) that are already contributing to airway closing during sleep. The neural mechanisms that regulate respiration in response to changes in blood levels of carbon dioxide are also altered during sleep in such a way that, during slow-wave sleep, reduced levels of arterial CO_2, a normal stimulation to breathing during wakefulness, are less well perceived. Recent work has identified the neurons involved in the control of breathing, mapped some of the connections of these neurons, and characterized some of their neurotransmitters. Understanding how sleep changes the physiology of neurons related to respiration will provide a mechanistic explanation for the pathology underlying sleep apnea syndrome. It may also point to pharmacologic interventions that could prevent expression of the disease.

Cardiovascular function also changes during sleep. The regularity of the heart rate in wakefulness is disturbed in slow-wave and REM sleep. Some of this variability is attributable to the sleep-induced changes in respiratory function; however, changes in heart rate variability are also seen in patients whose respiratory function in sleep is relatively undisturbed. The clinical importance of understanding the cardiovascular changes is highlighted by their application to sudden infant death syndrome (SIDS). This tragic disease is the major cause of infant death in the first year of life (not including the period just after birth). The victims of SIDS are otherwise healthy infants, aged 2 to 4 months, who die unexpectedly in their sleep. In a recent prospective study of 7,000 infants, electrocardiographic and chest-wall movements were

monitored nightly to identify any changes in cardiovascular or respiratory function that might predispose an infant to the disease. The results of this study indicated that infants who died of SIDS had an increased heart rate and decreases in certain forms of heart rate variation in sleep compared with normal infants. The mechanisms underlying the normal development of heart rate and respiration in different sleep states are just beginning to be studied and may involve interactions between the brainstem and a recently described group of neurons in the forebrain that appear to exert control over cardiac and respiratory functions during particular states. In addition, another important example of the relationship of body rhythms to cardiovascular disease has recently become evident in the increased occurrence of myocardial infarction (heart attacks) in the early morning.

Psychiatric Concomitants of Sleep

Patients with psychiatric disorders commonly show marked disorders of sleep, and a large number of patients with sleep disorders have accompanying psychiatric problems; the causal nature of this relationship (e.g., whether a sleep disorder causes the psychiatric symptoms or vice versa) is sometimes unclear. This link between psychiatric and sleep disorders is most apparent in one type of depression. Such patients generally show insomnia (though there is a subgroup that is hypersomnolent), a relative lack of deep slow-wave sleep in the early night, and, most remarkably, a stereotypic early-onset REM sleep (short REM latency) as well as increased ocular activity during REM sleep (increased REM density).

Two experimental approaches have been taken to explore the nature of this relationship: altering sleep in normal individuals in an attempt to generate depressive symptoms and altering sleep in depressed patients in an attempt to ameliorate the disease. The first approach has not been successful. Normal individuals whose sleep pattern is disrupted become dysphoric (uncomfortable), but not depressed (melancholic). The second approach, in contrast, has had remarkable success. Total or partial sleep deprivation of depressed patients has powerful antidepressant effects. Furthermore, in one classic study, selective deprivation of REM sleep for 2-3 weeks had significant antidepressant effects in patients with endogenous depression. However, the anti-depressant effects of briefer periods of total and partial sleep deprivation disappeared upon resumption of normal sleep patterns, limiting the clinical usefulness of sleep deprivation therapy. On the basis of animal experiments, stimulation of serotonin and norepinephrine systems has been postulated as an underlying mechanism for the anti-depressant effects of sleep deprivation, but much further work is needed to understand this clinically intriguing response. Recent preliminary data from positron emission tomography (PET) scan studies of brain glucose metabolism in awake depressed patients before and after sleep deprivation suggest that clinical improvement occurs in patients with elevated metabolic activity in the cingulate gyrus, a part of the brain's so-called limbic system that is strongly implicated in emotional responses. The clinical improvement in these patients is associated with a return of the metabolic activity in the cingulate gyrus to a pattern typical of non-depressed controls.

Novel Technologies in Sleep Research

Sleep research has benefitted in the past decade from use of new techniques that have been developed in the fields of neuroscience and cell biology. For example, in vivo voltammetry and in vivo dialysis are extremely sensitive methods for measuring the chemical activity of neurons. Specially designed probes can be placed in the brain and used to measure the levels of neurotransmitters in very small regions by electrical (voltammetry) and chemical (dialysis combined with radioimmunoassay) means. Use of such technologies will allow quantification of neurotransmitter release during various sleep states and analysis of the possible irregularities in release that might lead to narcolepsy or depression.

Dynamic brain imaging techniques such as PET scanning enable noninvasive visualization of energy metabolism and/or blood flow in the brain. This technique has recently been applied to the sleeping brain to identify particular neuroanatomical structures in which metabolism is markedly altered as a function of sleep state. In addition, metabolic studies of the brain in lower animals, utilizing the deoxyglucose technique, and in men, utilizing both cerebral blood flow and deoxyglucose methods, have shown that the overall metabolic rate of the brain declines about 25-30 percent during nonREM sleep compared with wakefulness. The metabolic rate during REM sleep is about the same or somewhat increased compared with wakefulness, but the functional organization of the brain is changed dramatically. Another imaging technology, magnetic resonance spectroscopy, will enable researchers to look at the utilization of specific neurochemicals (neurotransmitters, neuromodulators, and hormones) during the varied states of sleep and wakefulness. Such studies will address in a noninvasive manner the questions of function, control, and mechanism of sleep in living animals, including human beings.

The techniques of recombinant DNA technology also have direct application to research in sleep. Canine narcolepsy is genetically transmitted in several breeds of dogs[3]. Methods developed in the field of genetics, such as restriction fragment length polymorphism (RFLP) technology, may help to identify the mutant gene in these animals. RFLP is a means for determining the precise location of a mutant gene by detecting certain regions of chromosomes that migrate (during recombination or reproduction) with the gene for a particular disease. This technique has been used, for example, to clone the mutant gene responsible for muscular dystrophy. Identification of the gene responsible for canine narcolepsy would lead to greater insight into the molecular mechanism underlying the cellular physiology of both normal and diseased REM sleep.

Other recombinant technologies that offer great promise for basic sleep research include subtractive hybridization techniques, and the use of transgenic and homologous recombinant mice. Subtractive hybridization is a method for the identification of genes that are expressed at certain times and not at others. This technique has recently been applied to the study of sleeping and waking animal brains in the hope of identifying new molecules that alter the intrinsic properties of neurons and thereby generate the altered physiology of sleep. Transgenic mice are animals in which specifically engineered genes have been introduced into their chromosomes; homologous recombinants are mice in which specific endogenous genes have been specifically altered or removed. Gene products that are identified by subtractive hybridization, RFLP analysis, or other means can be evaluated for their effects on sleep and wakefulness. For example, using the homologous recombinant mouse, it is possible to investigate what happens to REM sleep if the acetylcholine receptor is mutated. Such powerful technologies can lead to the ultimate realization of reductionist behavioral neuroscience: to study the effects of a single molecule on the behavior of the whole animal.

Conclusions and Recommendations

Remarkable progress has been made in the past decade in understanding sleep. Ten years ago, the phenomenology of sleep was well described, but the control of sleeping and waking, and the neural mechanisms that generate the complex physiology of sleep were largely unknown. With the application of methodologies and principles from basic neurobiology, particularly the use of intracellular recordings, pharmacologic agents that mimic neurotransmitters, in vitro slice preparations, and selective ablation studies, the basic mechanisms and controls of sleep have been organized into a framework with great explanatory and predictive power. This understanding has already had direct application to the treatment of a number of highly prevalent diseases and disorders, including depression, insomnia, jet lag, sleep apnea syndrome, and narcolepsy.

In many ways, though, the study of sleep is just beginning. As has been evident throughout this report, sleep is not just a quiescent phase of our existence, but is made up of a complex set of active behavioral and physiological processes. The cellular physiological and biochemical substrates of sleep provide an accessible starting point for investigations aimed in two directions. First, following the advances in cellular and molecular biology that have occurred in the field of neuroscience, the roles of particular molecules in individual neurons that control and generate sleep are now accessible through the use of single cell neurophysiological and molecular biological techniques. Application of such techniques has already identified a number of neurotransmitters and neuromodulators whose functions are central to sleep; many more remain to be discovered and characterized before a coherent picture of the control and progression of sleep can be understood. Second, such reductionist work must be complemented by integrative neurobiology to generate a dynamic description of sleep. The timely importance of such a description is witnessed by the exponential growth of sleep disorder centers throughout the nation. The first such center opened fifteen years ago; today, there are over 2,000 such centers seeing hundreds of thousands of patients annually. Despite impressive progress, basic sleep research has not kept pace with the clinical needs of the growing number of patients seen in sleep disorder centers. This may be a result of there being fewer than 15 universities and other institutions with substantial research programs in basic sleep research in humans and animals and fewer than 20 tenured scientists pursuing this research. Effective and practical preventative measures and treatments are urgently needed for narcolepsy, sleep apnea syndrome, sudden infant death syndrome, insomnia, jet lag, depression, and daytime sleepiness in the elderly, and these can only come through increased basic knowledge about sleep.

To insure continued progress toward understanding

3 There is also evidence that there may be a genetic link in human narcolepsy, because the majority of narcoleptic patients exhibit a specific biochemical marker.

sleep, the panel has identified the following as among the key questions for future research:

- What are the functions of sleep and how do the physiological mechanisms of sleep subserve these functions?
- What are the biochemical and physiological mechanisms of the homeostatic and circadian control of sleep states?
- How does the circadian timekeeping system interact with the sleep-wake system in terms of neuroanatomy, neurophysiology, biochemistry, and molecular biology?
- What are the processes that underlie the effect of environmental light exposure on human circadian cycles?
- What cellular and molecular events are crucial to generation of sleep and to the modulation of intrinsic neuronal properties during sleep?
- What changes in neuronal activity and/or properties lead to changes in somatic functions such as respiration and heart rate?
- What are the physiological mechanisms of daytime sleepiness? To what extent are they under circadian versus homeostatic control?
- How are the many changes and alterations observed during sleep coordinated?
- What genes are essential to normal sleep and circadian regulation and how is gene expression changed during perturbations of normal sleep-wake cycles?

In addition to these basic scientific questions, the research briefing panel feels that sleep research should continue and be strengthened. The panel affirms and endorses the use of animals in basic sleep research as essential in providing knowledge leading to alleviation of human sleep disorders. Facilitating the transfer of information from basic research to the process of development of new therapies for sleep and related disorders is an important goal. To this end and for the generation of coherent theories of sleep function, the panel supports the encouragement of vertical integration of sleep studies, especially through collaborations between laboratories, so that basic molecular, cellular, and system functions in sleep can be studied. Such integration and collaboration might be supported by program project and center grants for basic sleep research. In addition, because sleep studies span a number of diverse disciplines, and because data from these studies are published in many different journals and books, the panel agrees that there is a need for a "sleep information service" to collate, integrate, and disseminate information on sleep processes. Finally, the panel feels that strategies should be developed to attract young basic and clinical investigators to sleep research and to provide training in the many new techniques useful in the field. Such strategies could include undergraduate courses in the neurobiology of sleep, development of graduate and post-doctoral training programs in sleep research, and encouragement of multi-disciplinary research opportunities for those interested in sleep. The inclusion of individuals with expertise in sleep research in the peer review process should also be encouraged.

READING LIST

The following sources are included for the interested reader who wishes to investigate further some of the areas covered in this research briefing. This is not a comprehensive bibliography, but it is intended to provide enough source material, such as is found in review papers, to lead the reader to more specialized reports and journal articles.

Kryger, MH, Roth, T, and Dement, WC, eds. *Principles and Practice of Sleep Medicine*. New York: Saunders, 1989.

Steriade, M and McCarley, RW. *Brainstem Control of Wakefulness and Sleep*. New York: Plenum Press, 1990.